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Prospective study of Human Bocavirus (HBoV) infection in a pediatric university hospital in Germany 2005/2006

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Abstract

Background: Human Bocavirus (HBoV), a new species of the genus parvovirus newly detected in 2005, seems to be a worldwide distributed pathogen among children with respiratory tract infection (prevalence 2%–18%). Recently published retrospective studies and one prospective birth cohort study suggest that HBoV-primary infection occurs in infants.
Methods: Prospective single center study over one winter season (November 2005–May 2006) with hospitalized children without age restriction using PCR-based diagnostic methods.
Results: HBoV DNA was detected in 11 (2.8%) of 389 nasopharyngeal aspirates from symptomatic hospitalized children (median age 9.0 months; range: 3–17 months). RSV, HMPV, HCoV, and Influenza B were detected in 13.9% ($n=54$), 5.1% ($n=20$), 2.6% ($n=10$), and 1.8% ($n=7$), respectively. There was no influenza A DNA detected in any of the specimens. The clinical diagnoses were acute wheezing (bronchitis) in four patients, radiologically confirmed pneumonia in six patients (55%) and croup syndrome in one patient. In five to six patients with pneumonia, HBoV was the only pathogen detected. While no patient had to be mechanically ventilated, 73% needed oxygen supplementation. In four (36.4%) patients at least one other viral pathogen was found (plus RSV $n=3$; 27.3%; Norovirus $n=1$; 9.1%).
Conclusion: HBoV causes severe respiratory tract infections in infants and young children. Its role as a copathogen and many other open questions has to be defined in further prospective studies.
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Keywords: Human Bocavirus; Respiratory tract infection; Hospitalized infants and children

1. Introduction

Human Bocavirus (HBoV) was first described by Allander et al. (2005) (Karolinska University Hospital, Stockholm) in 2005. The virus was identified in retrospectively analyzed clinical specimen from infants and children with respiratory tract infections (RTI). HBoV seems to be a worldwide distributed pathogen and has frequently been associated with respiratory tract infections among infants and young children. HBoV-positive patients also presented with symptoms of gastrointestinal disease (Arnold et al., 2006; Kesebir et al., 2006). HBoV DNA has recently been detected in stool samples of patients unrelated to respiratory infection (Vicente et al., 2007) raising questions about different possible modes of transmission.

Unlike patients with RSV-infection (Ogra, 2004; Simon et al., 2006), and similar to those with HMPV-associated respiratory tract infection (Wilkesmann et al., 2006), children older than 6 months seem to be most at risk (Kesebir et al., 2006; Manning et al., 2006; Weissbrich et al., 2006). To this point there have only been few reports of infection in adult populations (Bastien et al., 2006; Manning et al.,

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2006; Sloots et al., 2006). Detection of HBoV is only possible with RT-PCR (Allander et al., 2005). Modified real time RT-PCR protocols have been recently published (Kleines et al., 2007; Lin et al., 2007; Lu et al., 2006; Schenk et al., 2007) and detected 10^1–10^{10} copies/mL in respiratory secretions of symptomatic patients. Hitherto, no animal model and no method for virus culture are available.

As Koch's revised postulates are not yet fulfilled for HBoV well designed clinical studies should investigate the association between the virus and respiratory and possible gastrointestinal manifestation (Arnold et al., 2006; Kesebir et al., 2006). However, statistical significant association between HBoV detection and previously unexplained disease (Allander et al., 2007, 2005) and a significantly higher frequency of HBoV DNA detection in symptomatic than in asymptomatic individuals (Allander et al., 2007, 2005; Kesebir et al., 2006) support the idea of a possible association of virus and clinical symptoms. Co-infection with other viral pathogens is a frequently confirmed feature in HBoV infection raising the question of causality (Allander et al., 2007; Kaplan et al., 2006; Smuts and Hardie, 2006). At the Children's Hospital, University of Bonn, viral RTIs are prospectively investigated in a multicenter surveillance study since 1998 (Simon et al., 2006). This report discusses the prospectively documented viral RTIs in hospitalized pediatric patients in the 2005–2006 winter season and focuses on the HBoV infections detected. To put our results into perspective, we analyzed 17 publications (Allander et al., 2005; Arnold et al., 2006; Bastien et al., 2006; Choi et al., 2006; Foulongne et al., 2006a,b; Kaplan et al., 2006; Kesebir et al., 2006; Kleines et al., 2007; Lin et al., 2007; Lu et al., 2006; Ma et al., 2006; Manning et al., 2006; Regamey et al., 2007; Schenk et al., 2007; Sloots et al., 2006; Smuts and Hardie, 2006; Weissbrich et al., 2006) dealing with HBoV-infection.

2. Methods

2.1. The clinical database-VIRTI Ped

In November 1999 a specific software tool developed at our institution for the targeted surveillance of hospitalized RSV-infected pediatric patients was made available for data entry. Inclusion criteria and details of the surveillance protocol have been published recently (Simon et al., 2007a, 2006). In 2004, the database was modified and reopened to clinical data entry considering other pathogens which cause viral respiratory tract infections (VIRTI Ped database) (Simon et al., 2007a,b; Wilkesmann et al., 2006). For the purpose of this prospective analysis, all pediatric inpatients (hospitalized from November 01, 2005 to May 31, 2006) with virologically confirmed RSV, HMPV, HCoV and HBoV infection were included irrespective of age (<18 years), underlying disease, comorbidity and whether the infection had been acquired in the hospital or at home. A lower respiratory tract infection was only documented as 'pneumonia' only if a chest radiograph confirmed the clinical diagnosis (Donnelly, 2001). The study protocol of the VIRTI Ped Study was approved by the ethics committee of the University of Bonn. Before enrolment and data entry into the database, informed consent was obtained from parents or the patients' legal guardians.

3. Virological methods

Nasopharyngeal specimen from patients suffering from respiratory illness were tested as previously described for the following viruses: RT-PCR for human coronaviruses (NL63, SARS, HKU1, OC43, 229E) as described below, RT-PCR for human metapneumovirus (Schildgen et al., 2004; Viazov et al., 2003; Wilkesmann et al., 2006), RT-PCR and NOW® RSV ELISA (Inverness Medical, Cologne, Germany) for respiratory syncytial virus (Simon et al., 2006), human influenza viruses A and B and NOW® Influenza A/B ELISA (Inverness Medical, Cologne, Germany), and Human Bocavirus (Allander et al., 2005; Kupfer et al., 2006; Simon et al., 2007b). Except for human coronaviruses all methods were described in detail previously (Allander et al., 2005; Kupfer et al., 2006; Schildgen et al., 2004; Simon et al., 2006; Viazov et al., 2003; Wilkesmann et al., 2006).

For the amplification of HCoV-specific sequences RNA was extracted from nasopharyngeal aspirates with the QIAamp MinElute Virus Spin Kit (QIAGEN, Hilden, Germany) and subjected to a single-round RT-PCR (QIAGEN, Hilden, Germany) using a set of primers designed on the basis of comparison of *pol* ORF1b sequences of known human coronaviruses (Kupfer et al., 2006). RT was carried out for 30 min at 42 °C followed by a PCR with one initial step of denaturation (95 °C for 5 min), 35 cycles of amplification (95 °C for 30 s, 50 °C for 30 s, 72 °C for 30 s) and a final elongation step (72 °C for 5 min). The primers used for the pan-coronavirus PCR were sv387as (5′-TCA CAY TTW GGA TAR TCC CA), sv388s (5′-ACT CAR ATR AAT CTT AAR TAY GC), and sv389s (5′-ACT CAA ATR AAT TTR AAR TAY GC) at final concentrations of 0.6 μM each. PCR-amplicons with a length of 251 basepairs were electrophoresed on an agarose gel and visualized by ethidium bromide staining. All positive PCR results, especially those that were positive for Human Bocavirus, were confirmed by sequencing.

Other common respiratory viruses such as human parainfluenza virus, human rhinoviruses or adenovirus were not included in the screening panel.

3.1. Sequencing of bocavirus PCR-amplificates

Amplicons were purified using the QIAquick PCR purification kit (Qiagen, Hilden, Germany) and subjected to sequencing in both directions (BigDye Terminator DNA sequencing kit v3.1, Applied Biosystems, Darmstadt, Germany) as previously described (Schildgen et al., 2004). BlastN (http://www.ncbi.nlm.nih.gov/BLAST) was used for

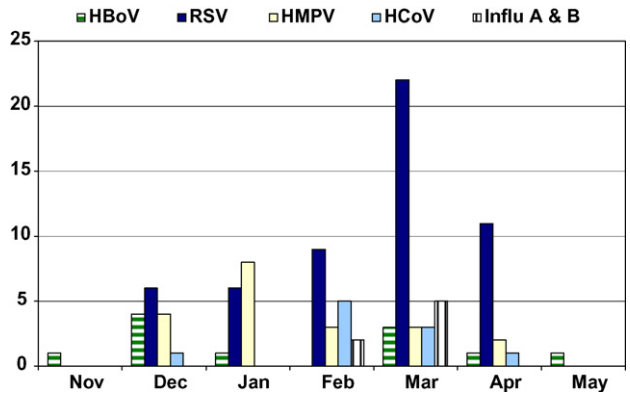

Fig. 1. Distribution of the positive specimens (absolute numbers) of the investigated viral respiratory pathogens in the corresponding months of prospective surveillance.

comparison of the obtained sequences to nucleotide sequence databases.

4. Results

From November 01, 2005 to May 31, 2006, 389 nasopharyngeal aspirates (NPA) from hospitalized pediatric patients with respiratory tract infection were investigated. Specimens from 2.8% ($n = 11$) of all patients were positive for HBoV. RSV, HMPV, HCoV, Influenza B viruses were detected in 13.9% ($n = 54$), 5.1% ($n = 20$), 2.6% ($n = 10$), and 1.8% ($n = 7$), respectively. There was no influenzavirus A detected.

Taken together, in 23.4% of all investigated children, at least one of the viral pathogens included into the diagnostic panel could be detected. Most patients with HBoV infection were found in December 2005 (Fig. 1). The proportion of the HBoV-positive specimens referring to all investigated specimens varied between 0.0% and 5.7% in the corresponding month of surveillance (Fig. 2). The median age of the HBoV-positive patients was 9.0 months (range, 3–17 months), eight

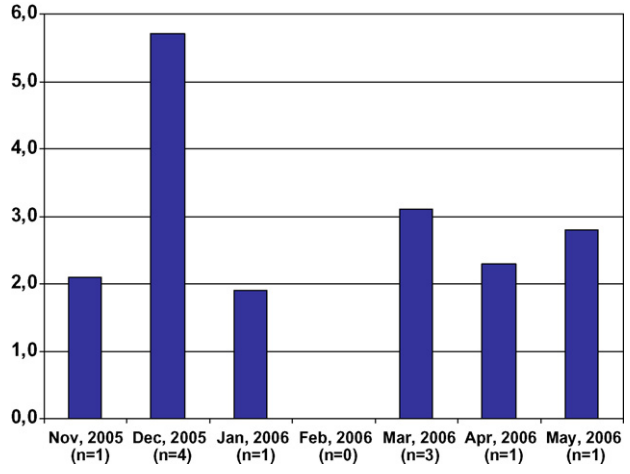

Fig. 2. Monthly distribution of the proportion of HBoV-positive specimens (in %) related to all tested nasopharyngeal aspirates.

(73%) were male. The median duration of symptoms before the admission to the hospital was 4 days (range 1–23 days), the median duration of hospitalization was 5 days (range 2–15 days). One patient had a preexisting comorbidity (premature birth, birth weight 980 g). The clinical diagnosis was acute wheezing (bronchitis) in four patients, pneumonia in six patients (55%) and croup syndrome in one patient. In eight (73%) HBoV-positive patients, the attending physicians ordered a chest radiograph, which yielded pathologic findings in seven patients (one peribronchitis, four bronchopneumonias, two lobar pneumonias). In five of six patients with radiologically confirmed pneumonia, HBoV was the only pathogen detected. While no patient had to be mechanically ventilated, 73% needed oxygen supplementation. The median leukocyte count at diagnosis was $11.3 \times 10^9\,L^{-1}$ (range: $(6.7–16.7) \times 10^9\,L^{-1}$); the median C-reactive protein was 12.5 mg/L (normal < 0.3mg/L; range: < 0.3–114 mg/L). The patients received treatment with betamimetics (inhalative; 10/11; 91%), systemic steroids (6/11; 55%) and antimicrobial chemotherapy (9/11; 82%); it has to be considered, that only in those patients with RSV-coinfection, the attending physicians were aware of a viral pathogen at the time of the clinical event. Beside one patient, in whom HBoV was detected at two consecutive days in two respiratory specimens, no follow up investigations were performed. In 7 of 11 HBoV-positive patients no other pathogen was detected, in 4 (36.4%) at least one other viral pathogen was found (plus RSV $n = 3$; 27.3%; Norovirus $n = 1$; 9.1%).

5. Discussion

This report represents the first prospective investigation of hospitalized pediatric patients with HBoV infection. Although the proportion of HBoV-positive specimens (2.8%) in our study was relatively low (range in the literature 1.5% (Bastien et al., 2006) to 18.3% (Kaplan et al., 2006)), illness severity was remarkable with radiological confirmed pneumonia and need for oxygen supplementation in the majority of the patients. Another recently published prospective study detected HBoV in five (4.5%) of 112 infants with RTI in a birth cohort study in Switzerland (Regamey et al., 2007); all these infants had mild disease and none was hospitalized. So, to define the definite role of HBoV as a respiratory pathogen, prospective population-based multicentre studies will have to be performed.

5.1. Seasonality

Most authors observed a higher prevalence of HBoV-infection in the cold winter and spring months (Allander et al., 2005; Regamey et al., 2007; Smuts and Hardie, 2006). Choi et al. (Corea 2000–2005) (Choi et al., 2006) reported a relatively high occurrence of HBoV in late spring and early summer and Bastien et al. (2006) from Canada detected HBoV throughout the year with no apparent seasonal prevalence.

Our data was collected during the months November 2005 and May 2006 and therefore, cannot contribute to an evaluation of the incidence of HBoV during the warm months of summer and early fall. Nevertheless, it showed a steady incidence during the cold months of fall and winter (Fig. 2) in accordance with most authors (Allander et al., 2005; Arnold et al., 2006; Kesebir et al., 2006).

5.2. Prevalence

The prevalence of HBoV-infection (more precisely defined: the detection of HBoV-genome in respiratory secretions of symptomatic patients) among hospitalized children with respiratory tract infection in the analyzed studies ranged between 1.5% (Bastien et al., 2006) and 18.3% (Kaplan et al., 2006). The majority of children with HBoV-infection was younger than 24 months (Allander et al., 2005; Sloots et al., 2006; Smuts and Hardie, 2006). Two recently published studies included control groups of asymptomatic children (Kesebir et al., 2006; Manning et al., 2006). Kesebir and co-workers isolated HBoV DNA in 22 of 425 NPA specimens from symptomatic children while in none of the specimens from 96 asymptomatic children HBoV DNA could be detected. Only one of the 21 HBoV-positive patients in the Scottish study was an asymptomatic child (Manning et al., 2006).

5.3. Symptoms and clinical diagnoses

Clinical symptoms most frequently associated with Human Bocavirus infection include cough, rhinorrhea and fever up to 39.5 °C (Schenk et al., 2007). Gastrointestinal symptoms were described in up to 25% of all patients (Arnold et al., 2006; Kesebir et al., 2006).

Two of the HBoV DNA positive individuals (18%) presented with diarrhea. In one of the two patients norovirus was detected in a stool sample, whereas the other patient showed no other pathogen in stool specimens. However, our study was not designed to evaluate gastrointestinal disease.

Members of the parvovirus family described in cattle cause gastrointestinal symptoms. Considering the stability of the human parvovirus B19, long-term persistence of HBoV in the inanimate environment may foster a fecal-oral route of transmission via contaminated fomites. Further studies should consider testing stool samples for Human Bocavirus to confirm gastrointestinal manifestation. The most common clinical diagnoses of HBoV-positive patients included upper respiratory tract infection, bronchitis, bronchiolitis, pneumonia and exacerbation of asthma bronchiale.

This spectrum of diagnoses is in accordance with other viral respiratory tract infections. Similar to the situation in RSV- and in HMPV-infection (Williams et al., 2004) there is a lack of international consensus about the definition of certain diseases which are important in this clinical context (American Academy of Pediatrics, 2006; Smyth and Openshaw, 2006).

Two studies provided differing definitions (Arnold et al., 2006; Choi et al., 2006) whereas the 15 remaining publications do not describe their criteria. The percentage of ‘bronchiolitis’ within the diagnostic spectrum ranged between 3.2% (Weissbrich et al., 2006) and 46% (Foulongne et al., 2006a,b). Only 6 out of 17 publications (Allander et al., 2005; Arnold et al., 2006; Kesebir et al., 2006; Kleines et al., 2007; Ma et al., 2006; Schenk et al., 2007) stipulated radiological confirmation of any ‘pneumonia’. Similar to RSV (Weigl et al., 2003) or HMPV (Wilkesmann et al., 2006), there are no distinct clinical signs in HBoV-infected patients which allow an etiological diagnosis on clinical grounds alone.

5.4. Laboratory findings

Arnold et al. (2006) reported a median leukocyte count of $13.3 \times 10^9\,L^{-1}$; Ma et al. (2006) found a median leukocyte count of $13.1 \times 10^9\,L^{-1}$ and a median CRP of 0.6 mg/L (< 0.2–4.48) in HBoV-infected subjects. This confirms our findings, that elevated leukocyte and CRP-values may be found in HBoV-infected children.

5.5. Radiological findings

The high percentage of pathological findings on chest radiographs in our study is in accordance with the results of other clinical research groups, which ranged from 43% (Allander et al., 2005) to 83% (Foulongne et al., 2006a,b; Kleines et al., 2007). Allander and co-workers reported interstitial bilateral infiltrates in six of seven performed chest-X-rays. Obviously, HBoV does not only cause central pneumonia (Fig. 1) but also interstitial and lobar pneumonia with or without pleural effusion (Schenk et al., 2007). Neither clinical symptoms nor laboratory parameters nor radiological findings are sufficient to make a clear distinction between bacterial and viral cause of pneumonia in these patients (Korppi, 2003, 2002; McIntosh, 2002).

5.6. Coinfection

The results of our study show a coinfection rate of 36%. Such high percentage was also found in the majority of the analyzed studies which report coinfection rates of 18% (Allander et al., 2005) to 72% (Kaplan et al., 2006) with RSV being the most prevalent copathogen in most studies (Kleines et al., 2007). Manning et al. (2006) detected a significantly higher rate of coinfections in HBoV-positive patients (43% [23/53] vs. 17% [47/271] positive for another viral pathogen; $P < 0.001$). Differences in sensitivity of screening methods and the diagnostic panel for the detection of additional respiratory pathogens strongly affect coinfection rates. Our study’s respiratory virus panel did not include adenovirus, human parainfluenza virus and human rhinovirus which might have contributed to an even higher rate of coinfection.

5.7. Incubation period and nosocomial infection

Since the incubation period of HBoV-infection is unknown it is not possible to comment on the incidence rate of nosocomial infections. So far there have been neither studies on the tenacity of the virus nor the effect of commonly used disinfectant soap and solutions (Hota, 2004). Parvovirus B19 shows high tenacity and great resistance to commonly used disinfectants (Dowell et al., 1995). Due to the close relationship within the family of the Parvoviridae it is possible that HBoV shows similar characteristics. Kesebir and co-workers reported three patients (14%) with nosocomial HBoV-infection ($n = 22$). The infected infants (1, 4 and 6 months of age) had been hospitalized since birth. Sequencing of two isolates showed identical nucleotide sequences. Kleines et al. (2007) described three additional HBoV DNA positive patients, who developed respiratory symptoms after hospital treatment for other diseases for at least 4 weeks. Kesebir et al. (2006) reported three patients (14%) with nosocomial HBoV infection ($n = 22$). Vertical transmission has not been ruled out as the authors did not acquire NPAs from the parents. None of the patients described in our study was suspected to have a nosocomially acquired HBoV infection.

5.8. Treatment with antibiotics

In our centre 82% of HBoV-positive patients received antibacterial chemotherapy. Before HBoV was known, for the responsible physicians, children with HBoV-infection are mostly "RSV-negative patients with pneumonia".

The uncertain etiology of the clinical illness of respiratory infections may explain the excessive application of antibiotics.

5.9. HBoV and asthma bronchiale

Viral infection of the respiratory tract has frequently been associated with recurrent airway obstruction ('wheezing') among infants and asthma exacerbation among older children. In our patients 4 out of 11 presented with wheezing as the leading symptom of their respiratory infection. All recently published clinical studies on HBoV report asthma exacerbation as a clinical symptom in up to 27% of the HBoV-positive patients (Arnold et al., 2006; Foulongne et al., 2006a,b). Half of the patients in the study by Allander et al. (2005) presented asthma bronchiale as an underlying disease. Recently, Allander et al. (2007) detected HBoV in 49 of 259 (19%) children (median age 1.6 years) hospitalized with acute wheezing. A large proportion of the cases were mixed infections with other viruses, but HBoV was the only virus detected in 12 children (5%). High viral loads of HBoV were noted mainly in the absence of other viral agents, suggesting a causative role for acute wheezing. In addition, infections that had uncertain clinical relevance and low viral loads were prevalent. HBoV DNA was frequently detected in serum specimens obtained from patients with acute wheezing, suggesting systemic infection. These recent results suggest a model for HBoV infection in which high viral loads are potentially associated with respiratory symptoms and low viral loads indicate asymptomatic shedding. Therefore, quantitative polymerase chain reaction analysis may be important for additional studies of HBoV (Allander et al., 2007; Anderson, 2007).

5.10. HBoV and immunosuppression

Several clinical research groups reported HBoV-positive immunosuppressed/ immunodeficient patients in their studies (Arnold et al., 2006; Manning et al., 2006; Smuts and Hardie, 2006). Arnold et al. (2006) presented two pediatric patients with HBoV-infection after organ transplantation. Smuts and Hardie (2006) reported HBoV-infection in eight HIV-infected pediatric patients and Manning and co-workers included two infected non-specified immunosuppressed adult patients in their study (Arnold et al., 2006; Manning et al., 2006; Smuts and Hardie, 2006). There were no immunocompromised individuals among the HBoV DNA positive patients treated at our center during the winter season 2005/2006.

Our group has recently published the clinical case of a severe HBoV-infection of a 28-year-old female patient with malignant B-cell lymphoma (Kupfer et al., 2006). On admission the patient showed pancytopenia, high fever and clinical and radiological signs of pneumonia (reticulo-nodular infiltrates in the CT-scan of the thorax). Despite the application of antibiotics, antifungal agents and ganciclovir the patient presented persistent fever for 14 more days. HBoV was retrospectively detected as the sole pathogen in the nasopharyngeal aspirate of this high-risk patient. The patient was discharged after 41 days of hospitalization.

5.11. Open questions for further studies

As long as we do have only a limited number of studies comparing detection rates in symptomatic and asymptomatic people (Fry et al., 2007), and neither an animal model nor a reliable cell culture method (Anderson, 2007), it remains difficult to confirm Koch's postulates and prove the causative role of HBoV as a real pathogen.

How high is the rate of hospitalization in infected outpatients?

Fecal excretion of HBoV as shown by Vicente et al. (2007) adds new concern about the transmission of HBoV. Is HBoV transmitted via fecal contamination in addition to respiratory secretions?

Human Bocavirus is prevalent among children with acute wheezing and can cause systemic infection (Allander et al., 2007), raising the question whether there is a risk of parenteral transmission via blood products or blood contamination.

Also tenacity of HBoV has not been determined yet and its susceptibility to hospital grade disinfectants will have to be investigated.

Studies from the United States and Germany reported nosocomial infections (Kesebir et al., 2006). However, due to the limited number of investigated cases the true impact of HBoV as nosocomial infection remains uncertain. Its investigation requires additional prospective studies.

Co-infection is a frequent finding in HBoV DNA positive patients. It remains to be determined whether HBoV plays a role modifying the clinical presentation of other viral respiratory tract infections (RSV, HMPV).

To this point authors have collected very little data to evaluate the probability of bacterial superinfection in HBoV infection.

Having been subject to several studies concerned with pediatric populations HBoVs role as a pathogen in adult populations has still to be investigated. Is HBoV a clinically relevant pathogen among the elderly (COPD, patients >60 years of age) and among patients with underlying diseases and predisposing risk factors (immunodeficiency, chronic lung diseases, prematurity)?

Due to the retrospective manner of data collection of most of the performed studies no sufficient evaluation of the outcome of therapeutic interventions could be made to this point. Which therapeutic interventions are of confirmed benefit in children with severe HBoV infection? Prospective multicenter population based studies and much additional work in the virological laboratories will be necessary to clear up these points.

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